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Patentanmeldung Nr. Patent application No. Demande de brevet n°

03078253.6

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

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(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
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Method for preparing a modified host cell

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Method for preparing a modified host cell

The present invention relates to a method for preparing a modified host cell. It also relates to a method for the production of proteins, metabolites and cell biomass making use of said modified host cells.

Genetic modification of cells can be done either by classical strain improvement or by metabolic pathway engineering (MPE; Khetan and Hu (1999) In:*Manual of Industrial Microbiology Biotechnology* (Eds. Demain and Davies) 717-724). Classical strain improvement has been proven to be a powerful technology for introduction of (random) mutations and the subsequent selection of strains with new features (e.g. improved metabolite production, altered morphology or growth on a specific substrate). However, some mutations such as new functionalities, new pathways and selection markers cannot be introduced by classical mutagenesis. In order to attain these latter mutations, one has to apply MPE, relying on molecular biology, in particular the introduction of new genetic material into the host. In literature, many examples can be found illustrating successful applications of MPE (Crawford *et al.* (1995) *Biotechnology* **13**:58-62; Khetan *et al.* (1996) *Ann. N Y Acad. Sci.* **782**:17-24.). Moreover, using modern MPE technology it is possible to introduce several new features in one round (Ye *et al.* (2000) *Science* **287**:303-305).In all these cases new and/or additional genetic material has been introduced into the host strain.
The introduction of DNA into a cell can be done using a wide range of known technologies (e.g. electroporation, conjugation, particle bombardment, injection, etc). It depends on the organism used, which technology will be applicable.
The genes can be introduced on an artificial carrier, like plasmids, cosmids or chromosomes, or, alternatively, integrated into the host genome. Alternatively, non-inheritable features, like anti-sense oligonucleotides, short-hairpin RNAs, non-coding RNAs, proteins or metabolites, can be introduced in the host cell to trigger permanent or temporary modifications in the metabolism.
Afterwards, only the cells that have taken up the particular molecules need to be isolated.

In literature, almost exclusively DNA and RNA molecules are used to modify the metabolism of a cell, but in most cases these do not encode a protein with a selectable

feature, like resistance against a certain metabolite. And as most cells do not take up the molecules there is a need for an efficient selection procedure to select the recombinant cells.

Moreover, if episomal constructs or non-inheritable features are used, a constant selection pressure must be applied once the modified cells are selected. Otherwise, these features are gradually being lost from the dividing microorganism. This is done with the aid of so-called selection markers. This can be a gene that encodes a protein, which will give resistance to a certain metabolite (like an antibiotic or a high metal concentration) or will enable the cells to grow on a specific carbon source.

Even though these procedures are elegant, selective and widely applied, there are certain drawbacks, especially for industrial applications.

In most industrial processes it is not possible to apply a constant selection pressure using antibiotics.

Lacking the possibility of constant selection pressure by antibiotics, the use of episomal constructs is only applicable in a few industrial processes, wherein the growth conditions (mostly the composition of the media in the case of micro-organisms) will be the selective pressure. An example is the use of auxotrophic growth markers. To this end an organism first must be mutated to select strains with the necessary growth deficiencies. In the second round these growth deficiencies can be used as a selective pressure. An intact copy of the mutated feature is co-introduced with the heterologous genes on the same plasmid. When the right growth conditions are applied the cells can only grow when they contain this plasmid with the functional genes. This will put strict limitations on how a production process is run and on the variation in media optimization. This will in turn limit the applicability of this technology. Most industrial organisms do not contain any auxotrophic marker and are often polyploids (for example see: Hadfield *et al.* (1995) *Curr. Genet.* **27**:217-228), which makes it difficult to select auxotrophic derivatives. Therefore, dominant markers as hygromycin resistance are used to select transformants of industrial strains. This renders the newly derived strain with a piece of heterozygous DNA that limits new transformation rounds. Moreover, in plant species it has been shown that these elements might cause instabilities (for example see: Windels et al. (2001) *Eur Food Res Technol* **213**:107-112).

Also, there is a limited set of selection markers available for each organism (see for example: Van den Berg and Steensma (1997) *Yeast* **13**:551-559), which will hamper the possibilities of this approach.

More widely used in industrial strains is the stable integration of new DNA in the genome. In most cases this is done by co-integration of the selection marker (US patent No. 6051431). In the first screen one selects the strains that contain the selection marker, and in the second screen one selects the strains also containing the gene(s) of interest. The major drawback is that the marker is also stably integrated in the genome, and the same marker cannot be used for a second transformation round. Two further drawbacks are that there is a need for two successive selection/screening rounds and that more DNA then necessary is being introduced. This additional DNA-load can be a burden for the cell (see for example: Elmore et al. (2001) *Agronomy J.* **93**:408-412). But more important, the ongoing public debate on correlation between the use of antibiotic resistance markers in industrial processes and the increase in natural antibiotic resistance makes alternatives essential. Apart from the examples mentioned above, there are many alternative tools available in which the selection marker can be removed form the genome after stable integration of the gene(s) of interest (see for examples: Lyznik *et al.* (1995) *Plant J.* **8**:177-186; Selten *et al.* (1997) US patent No. 6051431; Yang and Hughes (2001) *Biotechniques* **31**:1036-1041). However these are time consuming and can cause additional instabilities to the cell-line of interest. Moreover, in most cases (for example see: Lyznik *et al.* (1995)) there is still some DNA left on the chromosome.

As the optimization of (industrial) organisms is highly dependent on recombinant DNA techniques for introduction of new and/or more efficient metabolic pathways to the host strain, there is a need for a new technology. So far, selection of new strains with DNA integrated into the host genome, always involves the co-integration of a selectable marker, which limits the rate of new transformation rounds using these derivative strains. The present invention solves this problem by using non-inheritable markers or non-gene encoding markers. Cells, which have taken up these markers can be separated from the marker-free cells and subsequently be inoculated in fresh medium. After cell division the transformants derived in this way loose the marker and are marker-free as a rule. Subsequently, the integrated gene(s) of interest will result in the altered capabilities of the production organism. Furthermore, the transformants can directly be used for new transformation rounds.

According to one embodiment the method for preparation of a modified host cell according to the present invention comprises the steps of (a) transfecting a host cell with at least one compound of interest to which a label is covalently coupled and (b) isolating

the transfected host cell, wherein the label provides to the host cell a non-inheritable trait.

According to a further embodiment the present invention relates to a method for preparation of a modified host cell comprising the steps of (a) transfecting a host cell with at least one compound of interest to which a label is covalently coupled and (b) isolating the transfected host cell, wherein the label provides to the host cell a non-inheritable trait and wherein isolation of the transfected host is established by direct separation of the host cells containing said label from host cells not containing said label.

According to a further embodiment the present invention relates to a method for preparing a modified host cell comprising the steps of (a) transfecting a host cell with at least one compound of interest covalently coupled to a label which provides to the host cell a non-inheritable trait, and (b) isolating the transfected host cell containing the label as obtained in step a) by using means that can distinguish and separate said transfected host cell containing said label from non-transfected host cell.

Subsequent to said separation step the transfected host cell can be cultured in order to multiply the organisms.

With the "compound of interest" is meant according to the present invention any compound, which enables to change permanently, or transiently a metabolic property of the host cell. Examples of "compounds of interest" are polynucleotides (for example nucleotide fragments such as a gene, a promoter, an expression cassette, a terminator, a plasmid, a small oligonucleotide which is able to interfere with a mRNA, RNA, hexose nucleic acid (HNA), peptide nucleic acid (PNA), or locked nucleic acid (LNA), antisense oligonucleotide, short hairpin RNA, non-coding DNA), proteins or metabolites.

The modified host cell can be used to produce oligonucleotides (for example RNA), (recombinant) proteins (for example antibodies, proteases, lipases, chymosine), primary or secondary metabolites (for example anti-infectives such as β-lactam antibiotics and building blocks, amino acids, and clavulanic acid), or biomass (such as yeast cells) on a laboratory or an industrial scale, for screening or commercial purposes, respectively.

With "modified host cell" according to the present invention is meant a host cell, which is permanently or transiently changed in its composition of DNA, RNA, proteins and/or metabolites. Such change might be established by over-expression of one or more gene(s); or by suppression of the expression of one or more gene(s); or by knocking out one or more gene(s); or by altering the regulation of one or more gene(s); or by gene

silencing of one or more gene(s); or by so-called RNA interference, whereby the messenger RNA levels of one ore more gene(s) are decreased or abolished; or by enzymes that trigger a response or divert cellular metabolism; or by metabolites that trigger a response or divert cellular metabolism. Preferably, the modified host according to the present invention expresses an additional gene (or genes) as compared to the unmodified host; or in the modified host a gene (or genes) is inactivated by deletion or interruption of said gene(s); or by binding of a complementary nucleotide sequence to said gene (or genes) or to part of said gene (or genes); or a combination of these methods. In the modified host according to the present invention DNA expression levels and/or RNA expression levels and/or protein expression levels and/or metabolite levels may be temporarily or structural altered.

"Transfecting a host" comprises transferring the at least one compound of interest, and optionally also other elements to provide stability to the host cell, or a secondary compound of interest, or an element for integration into the host cell.

The "label" according to the present invention comprises any label that is directly detectable or that can be made detectable. Preferably, the label is a fluorescent label, a luminescent label, a chemoluminescent label, an enzymatic label, a magnetic label, an antigenic label or a radioactive label.

Suitable fluorescent labels are rhodamine, fluorescein, alexa fluor, cascade blue, tetramethylrhodamine, texas red.

Suitable enzymatic labels include alkaline phosphatase, luciferase and ß-galactosidase.

Suitable luminescent labels include acridinium esters, luminol, isoluminol, oxalate esters, dioxetanes, and luciferin.

Suitable chemoluminescent labels include reactive aminoallyl-modified dNTPs, nitro blue tetrazolium, lucigenin.

Suitable radioactive labels include ^{3}H, ^{32}P, ^{15}N, ^{13}C, ^{14}C.

Suitable magnetic labels include magnetic beads containing iron, nickel or cobalt.

Suitable antigenic labels include FITC, digoxin and biotin. For proper detection these antigenic labels can be reacted with conjugated antibodies (such as anti-digoxin), or with conjugated streptavidine.

A "non-inheritable trait" (as opposed to an inheritable trait) means a structural or functional characteristic, which upon multiplication of the host will not be multiplied itself and subsequently will be diluted in the progeny of the host.

A suitable host cell according to the present invention is a prokaryotic organism, such as bacteria, or a eukaryotic organism such as yeast, fungi, plant cells or animal cells.
With "means to monitor and separate the transfected host" is meant any equipment or treatment, which may enable detection of the label covalently bound to the transfecting compound on single cell level and which converts the detected label to a signal that can be monitored. Subsequently, the means to monitor the label can also separate the single cell that contains the label from cells that do not contain said label.
In case the label is fluorescent or luminescent, these labels may be detected by using any apparatus that monitors electromagnetic radiation, such as infrared or ultraviolet light, X-rays, microwaves, and visible light. In case the label is a fluorescent label, the means to monitor said label may be a fluorescence detection apparatus. Preferably, it may be a Fluorescent Activated Cell Sorter (FACS).
In case the label is a radioactive label, the means to monitor said label may be a radiation detection apparatus such as a Geiger Muller teller or ^{13}C-NMR with cell sorting function.
In case the label is a (chemo)luminescent label, the means to monitor said label may be a FACS adapted with an additional luminometer.
In case the label is enzymatic, the means to monitor said label may be a FACS.
In case the label is magnetic, the means to monitor said label may be an autoMACS™.
The present invention also relates to a method for the preparation of a desired compound by a transformed host cell comprising the steps of a) transfecting a host with at least one polynucleotide involved in the production of said desired compound and which is covalently coupled to a label which provides to the host cell a non-inheritable trait, b) isolating the transfected host, c) culturing the transfected host under proliferating conditions, d) culturing the transfected host under conditions wherein the desired compound is produced and e) isolating the desired compound from the culture broth.
In a preferred embodiment the polynucleotide modifies the titer, stability, isolation and/or activity of said desired compound. Preferably the desired compound so produced is a protein, more preferably an enzyme.
The present invention also relates to a method for the preparation of a desired metabolite by a transformed host cell comprising the steps of a) transfecting a host cell with at least one polynucleotide involved in the production of said desired metabolite and which is covalently coupled to a label which provides to the host cell a non-inheritable trait, b) isolating the transfected host cell, c) culturing the transfected host cell under

proliferating conditions, d) culturing the transfected host cell under conditions wherein the desired metabolite is produced, and e) isolating the desired metabolite from the culture broth.

In a preferred embodiment of this latter method the polynucleotide is selected from the group consisting of DNA, RNA, short hairpin RNA, non-coding RNA, LNA, HNA and PNA.

In a further embodiment of this method the polynucleotide modifies the cellular metabolism via redirecting metabolic fluxes towards said metabolite. Preferably, the desired metabolite is a primary metabolite such as an amino acid, a steroid or a nucleotide. More preferably, the desired metabolite is a secondary metabolite, such as an antibiotic, a vitamin, an anti-infective, a macrolide, a polyketide, a pheromone, an alkaloid or a drug.

The present invention also relates to a method for the preparation of a desired biomass from a transformed host cell comprising the steps of a) transfecting a host cell with at least one polynucleotide involved in the production of said desired biomass and which is covalently coupled to a label which provides to the host cell a non-inheritable trait, b) isolating the transfected host, c) culturing the transfected host under proliferating conditions, d) culturing the transfected host under conditions wherein the desired biomass is produced, and e) isolating the desired biomass.

Advantageously, the desired biomass is a yeast cell. In another embodiment the desired biomass is a biocatalyst. In a further embodiment the desired biomass comprises sreenable cells for drug discovery.

Description of the figures

Fig. 1) Cells of *Penicillium chrysogenum* transfected with purified enhanced Green Fluorescent Protein.

A) light microscopy;

B) fluorescence microscopy (excitation at 460 nm; emission at 525 nm).

Fig. 2) Cells of *Penicillium chrysogenum* transfected with fluorescent oligonucleotides.

A) light microscopy;

B) fluorescence microscopy. (excitation at 460 nm; emission at 525 nm)

Fig. 3) Cells of *Penicillium chrysogenum* transfected with fluorescent Dextran 10.000 MW. A combination of light and fluorescence microscopy. (excitation at 460 nm; emission at 525 nm)

Fig. 4) Fluorescence activated cell sorter profiles from *Penicillium chrysogenum* protoplasts (excitation at 460 nm; emission at 525 nm).

A) Non-transfected protoplasts;

B) Protoplasts stained with vacuolar marker MFY-64 (Molecular Probes);

C) Protoplasts transfected with unlabeled pAMPF7;

D) Protoplasts transfected with fluorescently labelled pAMPF7.

Fig. 5) Cells of *Penicillium chrysogenum* transfected with fluorescently labelled plasmid pAMPF7.

A) Light microscopy;

B) Fluorescence microscopy (excitation at 460 nm; emission at 525 nm).

Examples

Example 1

Transfection of with Green Fluorescent Protein

Penicillium chrysogenum was cultivated for 48 hours in standard glucose medium. Cells were washed and cell walls were degraded using 4 mg/ml Novozym (NOVO/Nordisk). The obtained protoplasts were washed twice and used for transfection as described by Theilgaard *et al.* (2001, *Biotechnol. Bioeng.* **72**:379-388). During transfection 20 nM of purified, recombinant Enhanced Green Fluorescent Protein (Clontech; cat# 8365-1) was added. After transfection protoplasts were washed twice with SCT (Sorbitol, 1.2 M; $CaCl_2$, 50 mM; Tris-HCl, 10 mM; pH 7.5). Several competent cells were shown to have taken up the fluorescent protein (see Figure 1).

This example demonstrates that applying directly detectable signals (in this case fluorescence) covalently linked to a protein as a means of selecting cells results in desired cells in which that protein could trigger a (transient) metabolic change.

Example 2

Transfection of cells with fluorescently labelled DNA oligonucleotides

Competent *P. chrysogenum* protoplasts were obtained and processed as described in example 1. Two complementary, fluorescently labeled oligonucleotides were used: fluorescein-5'-GGGGAATGGACAAAA-3' and fluorescein-5'-TTTTGTCCATTTCCCC-3' (Life Technologies). Respectively 38 and 48 nmol of both oligonucleotides were added to the transfection mixture (according to Theilgaard *et al.* (2001)), resulting in a final concentration of 46 µM during the actual transfection. After transfection several fluorescent cells could be visualized (see Figure 2).

This example demonstrates that applying directly detectable signals (in this case fluorescein) covalently coupled to oligonucleotides as a means of selecting desired cells results in cells in which those oligonucleotides can trigger (transient) metabolic changes.

Example 3

Transfection of cells with fluorescently labeled Dextran

Competent *P. chrysogenum* protoplasts were obtained and processed as described in example 1. A fluorescently labeled hydrophilic polysaccharide, Dextran 10.000 MW (Molecular Probes, cat# D-7169) was used in a final concentration of 50 µM during the actual transfection. After transfection several fluorescent cells could be visualized (see Figure 3).

This example demonstrates that applying directly detectable signals (in this case fluorescence) covalently coupled to a compound as a means of selecting desired cells results in cells in which those compounds could trigger (transient) metabolic changes.

Example 4

Transfection of cells with fluorescently labeled DNA

Transfection

Competent *P. chrysogenum* protoplasts were obtained and processed as described in example 1. Five µg of plasmid pAMPF7, containing an expression cassette to drive expression of the enzyme acetamidase in the fungus (Fierro *et al.* (1996) *Curr Genet.* **29**:482-489), was labeled using the Label IT Nucleic acid labeling reagent (Mirus; cat# MIR3200). The label, 5-carboxy fluorescein, was attached to the guanine residues of the plasmid according to the supplier's manual. The sample was purified using the purification column. The efficiency of labeling was checked on agarose gels. One µg of fluorescently labeled plasmid was used for transfection. Cells were washed once with SCT and used directly after for selection, First on fluorescence and secondly on agar plates containing acetamide as the sole nitrogen source.

Selection for fluorescent cells using a Fluorescence Activated Cell Sorter (FACS)

A Fluorescence Activated Cell Sorter was used for isolation of the transfected cells (as described by De Nooij-Van Dalen *et al.* (2001) Genes Chromosomes Cancer **30**:323-335). After transformation approximately 10^6 cells were re-suspended in 4 ml of STC. Sorted cells were collected in STC with 5% BSA (w/v). As controls non-transfected protoplasts were sorted (see Figure 4A), protoplasts stained with the fluorescent vacuolar marker MDY-64 (see Figure 4B) and protoplasts transfected with unlabelled pAMPF7 (see Figure 4C). It was possible to obtain at least a 100-fold difference in labeled and unlabelled protoplasts (compare Figs. 4A and 4B). Therefore, we concluded that using this method we could isolate transfected cells with a fluorescent DNA molecule. This is showed in Figs. 4C and 4D. Using unlabelled pAMPF7 most cells show the same fluorescence as non-transfected protoplast. There is a small peak visible at a higher relative fluorescence (around 6-7x10^2), but this was due to the clumping of some cells after the polyethyleneglycol-mediated transfection. This level was set as a threshold value for the selection of fluorescent cells in the experiment with labeled pAMPF7. We were able to select approximately 3200 cells that showed a relative high fluorescence (around 6-7x10^3; see Figure 4D).

Fluorescence of selected cells

A sample of the 3200 cells selected was used for fluorescence microscopy. Figure 5 shows that a bright fluorescence is visible within these cells.

Selection for presence of functional amdS gene

Another sample of the 3200 cells selected were plated on media with acetamide as the sole nitrogen source. After a week several colonies were obtained on this media. Showing that the label did not effect the functionality of the *amdS* gene and that we were able to select stable transformants solely on the presence of fluorescence. This example demonstrates that applying directly detectable signals (in this case fluorescein) covalently coupled to DNA as a means of selecting and sorting the desired, modified cells results in cells in which the polynucleotide of interest triggers permanent metabolic changes.

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CLAIMS

1) A method for preparation of a modified host cell comprising the steps of
   a) transfecting a host cell with at least one compound of interest to which a label is covalently coupled
   b) isolating the transfected host cell
   characterized in that the label provides to the host cell a non-inheritable trait.

2) Method for preparation of a modified host cell comprising the steps of
   a) transfecting a host cell with at least one compound of interest to which a label is covalently coupled
   b) isolating the transfected host cell
   characterized in that the label provides to the host cell a non-inheritable trait and isolation of the transfected host cell is established by direct separation of the host cells containing said label from host cells not containing said label.

3) A method for preparing a modified host cell comprising the steps of
   a) transfecting a host with at least one compound of interest covalently coupled to a label which provides to the host cell a non-inheritable trait, and
   b) isolating the transfected host cell containing the label as obtained in step a) by using means that can distinguish and separate said transfected host cell containing said label from non-transfected host cells.

4) A method according to claim 1 to 3, wherein the transfected host cell of step b) is subsequently cultured.

5) A method according to claim 1 to 3 wherein the compound of interest is a compound able to change permanently or transiently a metabolic property of the host cell.

6) A method according to claim 1 to 3 wherein the compound of interest is selected from the group consisting of polynucleotides, proteins and metabolites.

7) A method according to claim 1 to 3, wherein the label is selected from the group consisting of a fluorescent label, a luminescent label, a chemo-luminescent label, a magnetic label, an antigenic label, an enzymatic label or a radioactive label.

8) A method according to claim 3, wherein the label is a fluorescent label and the means for detection is a Fluorescent Activated Cell Sorter (FACS).

9) A method according to claim 1 to 3 wherein the modified host cell is a prokaryotic cell, an eukaryotic cell, a mammalian cell or a plant cell.

10) A method for the preparation of a desired compound by a transformed host cell comprising the steps of

a) transfecting a host with at least one polynucleotide involved the production of said desired compound and which is covalently coupled to a label which provides to the host cell a non-inheritable trait
b) isolating the transfected host
c) culturing the transfected host under proliferating conditions
d) culturing the transfected host under conditions wherein the desired compound is produced
e) isolating the desired compound from the culture broth.

11) A method according to claim 10 wherein the polynucleotide is selected from the group consisting of DNA, RNA, short hairpin RNA, non-coding RNA, LNA, HNA, PNA.

12) A method according to claim 10 wherein the polynucleotide modifies the titer, stability, isolation and/or activity of said desired compound

13) A method according to claim 10 wherein the desired compound is a protein.

14) A method according to claim 10 wherein the desired compound is an enzyme.

15) A method for the preparation of a desired metabolite by a transformed host cell comprising the steps of

a) transfecting a host cell with at least one polynucleotide involved in the production of said desired metabolite and which is covalently coupled to a label which provides to the host cell a non-inheritable trait
b) isolating the transfected host cell
c) culturing the transfected host cell under proliferating conditions
d) culturing the transfected host cell under conditions wherein the desired metabolite is produced
e) isolating the desired metabolite from the culture broth.

16) A method according to claim 15 wherein the polynucleotide is selected from the group consisting of DNA, RNA, short hairpin RNA, non-coding RNA, LNA, HNA, PNA.

17) A polynucleotide for use in a method according to claim 15, which modifies the cellular metabolism via redirecting metabolic fluxes towards said metabolite.

18) A method according to claim 15, wherein the desired metabolite is a primary metabolite.

19) A method according to claim 15, wherein the desired metabolite is an amino acid, a steroid or a nucleotide.

20) A method according to claim 15, wherein the desired metabolite is a secondary metabolite.

21) A method according to claim 15, wherein the desired secondary metabolite is an antibiotic, a vitamin, an anti-infective, a macrolide, a polyketide, a pheromone, an alkaloid or a drug.

22) A method for the preparation of a desired biomass by a transformed host cell comprising the steps of
a) transfecting a host cell with at least one polynucleotide involved in the production of said desired biomass and which is covalently coupled to a label which provides to the host cell a non-inheritable trait

b) isolating the transfected host
c) culturing the transfected host under proliferating conditions
d) culturing the transfected host under conditions wherein the desired biomass is produced
e) isolating the desired biomass.

23) A method according to claim 22 wherein the polynucleotide is selected from the group consisting of DNA, RNA, short hairpin RNA, non-coding RNA, LNA, HNA, PNA.

24) A method according to claim 22, wherein the desired biomass is a yeast cell.

25) A method according to claim 22, wherein the desired biomass comprises a biocatalyst.

26) A method according to claim 22, wherein the desired biomass comprises sreenable cells for drug discovery.

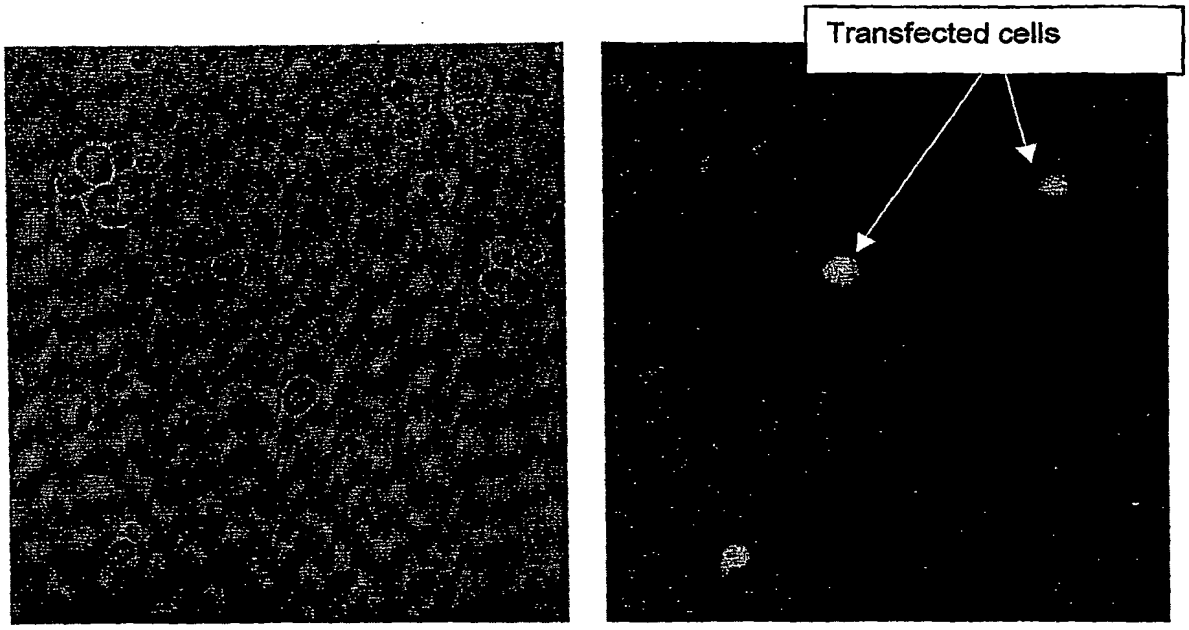
ABSTRACT

The present invention relates to a method for preparation of a modified host cell which comprises the steps of (a) transfecting a host cell with at least one compound of interest to which a label is covalently coupled and (b) isolating the transfected host cell, wherein the label provides to the host cell a non-inheritable trait. Modified host cells according to the invention can be directly separated from the non-modified host cell. To this end use is made of labels, which can be monitored at the modified cells (such as fluorescent labels) and which enable separation of the modified and non-modified host cells by suitable means. In case of fluorescent labels use can be made of a Fluorescent Activated Cell Sorter.

Suitable compounds of interest according to this invention are compounds, which enable to change permanently or transiently a metabolic property of the host cell. Examples of compounds are polynucleotides, proteins or metabolites.

The host cells modified according to the present invention can be used for the production of proteins, metabolites and cell biomass.

Fig.1/5.



A B

Fig.2/5.

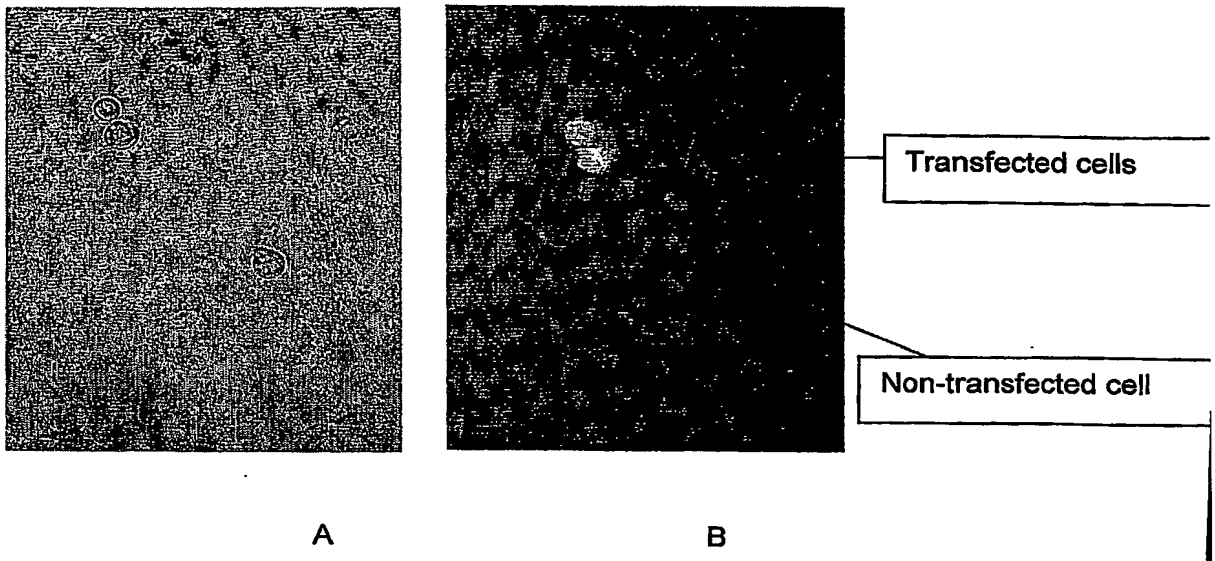


Fig.3/5.

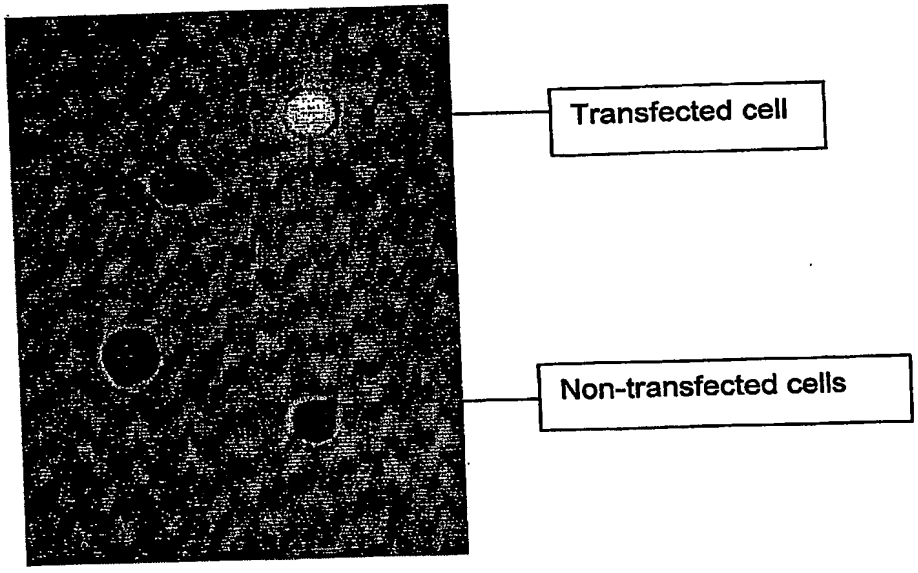
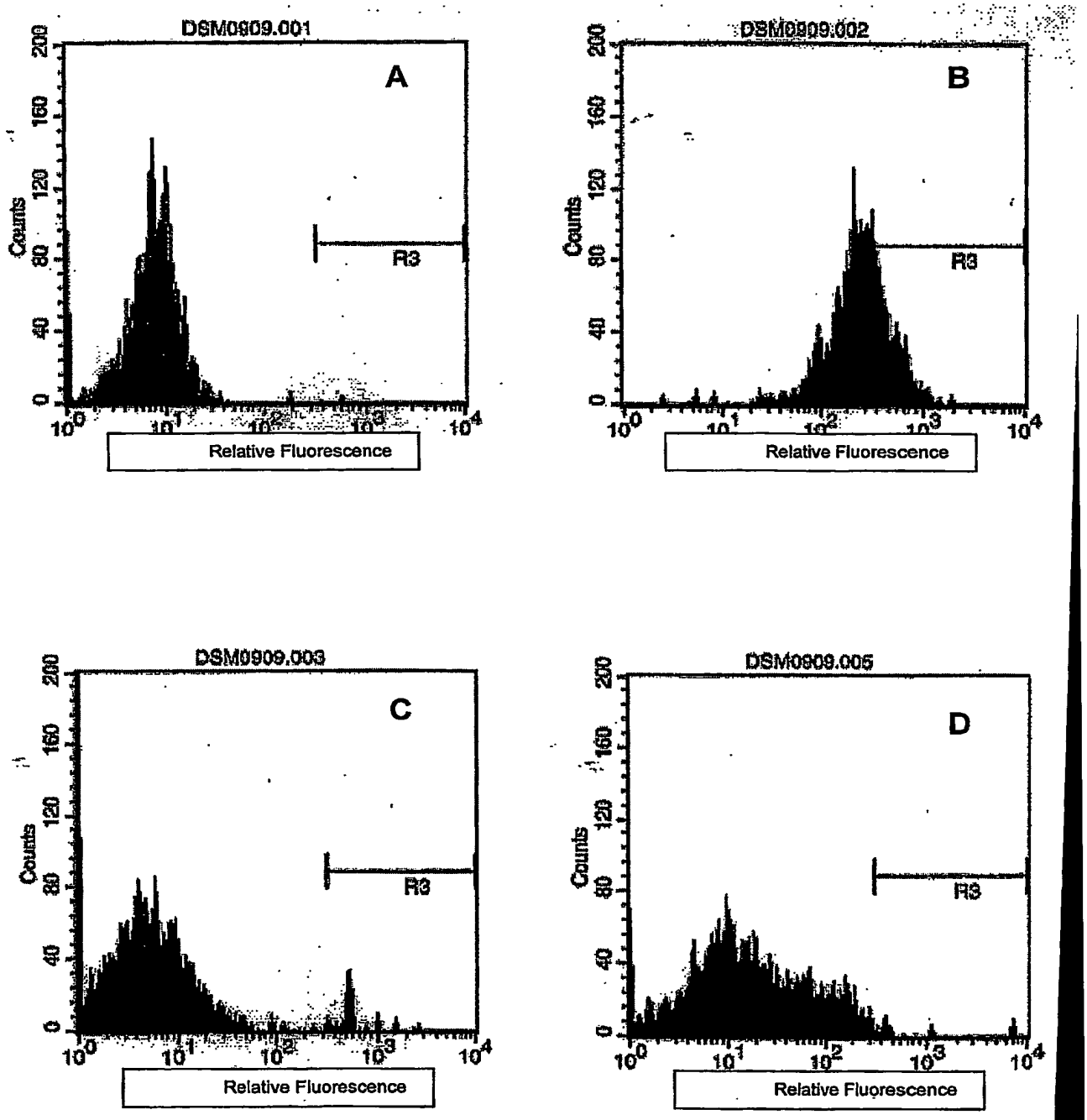


Fig.4/5.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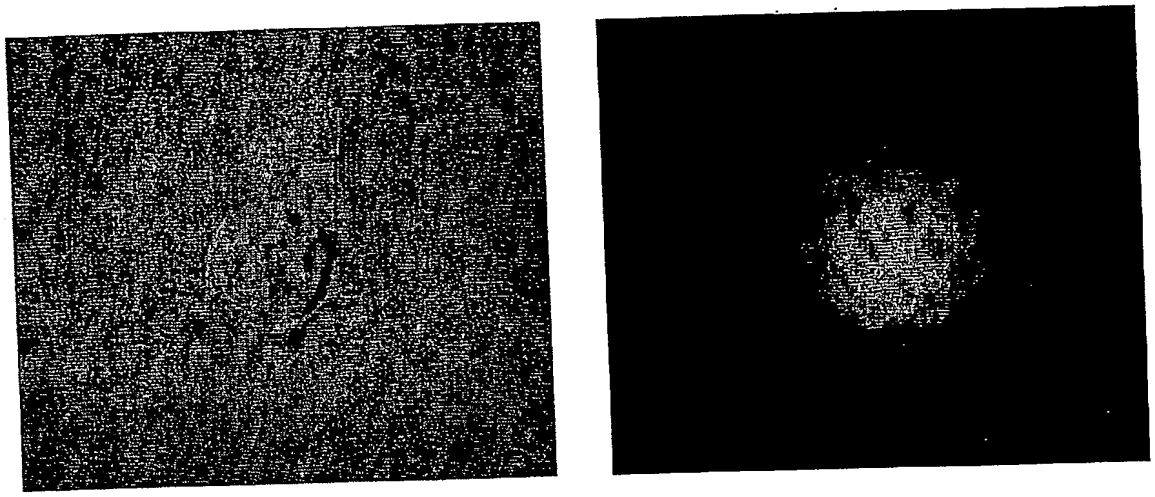
Relative Fluorescence

Fig.5/5.



A

B

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